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Blunted coronary vasodilator response to uridine adenosine tetraphosphate in post-infarct remodeled myocardium is due to reduced P1 receptor activation

Zhichao Zhou[a], Daphne de Wijs-Meijler[a], Inge Lankhuizen[a], Joachim Jankowski[c], Vera Jankowski[c], A.H. Jan Danser[b], Dirk J. Duncker[a], Daphne Merkus[a,*]

[a] Division of Experimental Cardiology, Department of Cardiology, Thoraxcenter, Cardiovascular Research School COEUR, The Netherlands
[b] Division of Pharmacology, Department of Internal Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands
[c] Charité-Universitaetsmedizin Berlin, Medizinische Klinik IV (CBF), Berlin, Germany

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ABSTRACT

We previously demonstrated that uridine adenosine tetraphosphate (Up_4A) exerts a potent vasodilator effect in the healthy porcine coronary vasculature. Since the coronary microvascular effects of Up_4A after myocardial infarction (MI) are unknown, the present study investigated the response to Up_4A in coronary microvessels from post-MI remodeled porcine myocardium, and the involvement of purinergic receptor subtypes. Coronary small arteries (diameter ~150 μm) were dissected from the apex of Sham-operated swine and swine in which MI had been produced 5 weeks earlier by transient (2 h) occlusion of the left circumflex coronary artery, and mounted on Mulvany wire myographs. Up_4A (10^{-9}–10^{-5} M) produced coronary vasodilation that was reduced in MI as compared to Sham-operated swine. Up_4A-induced vasodilation was reduced by P1 blockade with 8-phenyltheophylline in Sham-operated swine and to a lesser extent in MI, while the attenuation by the A_{2A} receptor blocker SCH58261 was similar in Sham-operated and MI swine. Up_4A-induced vasodilation remained unaffected by non-selective P2 receptor antagonist PPADS, but was attenuated by selective $P2X_1$ and $P2Y_1$ receptor antagonists MRS2159 and MRS2179, albeit to a similar extent in Sham-operated and MI swine. These responses were paralleled by similar mRNA expression levels of A_{2A}, $P2X_1$ and $P2Y_1$ receptors in MI compared to slaughterhouse control swine. Finally, attenuation of Up_4A-induced coronary vasodilation by nitric oxide synthase inhibition was not attenuated in MI as compared to Sham-operated swine. In conclusion, blunted coronary vasodilation in response to Up_4A in MI swine is most likely due to reduced activation of P1, rather than P2, receptors and does not involve a loss of NO bioavailability.

1. Introduction

The endothelium releases a variety of vasodilators, such as nitric oxide (NO) and prostacyclin, and vasoconstrictors, such as endothelin and reactive oxygen species [1]. A novel endothelium-derived vasoactive factor uridine adenosine tetraphosphate (Up_4A) has been recently identified [2]. Up_4A contains one purine and one pyrimidine moiety and therefore can exert its vasoactive effects through the purinergic P1 as well as P2 receptors [2–5]. These receptors are also involved in the vasoactive effects of purines such as adenosine, ATP and ADP as well as pyrimidines such as UTP and UDP. In contrast to the vasoconstrictor action of Up_4A in various vascular beds in rodents [3], we have recently shown that Up_4A exerts a potent vasodilator effect in the healthy porcine coronary vasculature that is largely mediated through P1 (A_{2A}) receptors [4] and partly involves the release of nitric oxide and prostacyclin from the endothelium. These observations suggest that Up_4A represents a novel vasodilator in the coronary microcirculation. Furthermore, the 10-fold elevation in plasma levels of Up_4A observed in juvenile hypertensive subjects [6], as well as the observation that the vasoconstriction to Up_4A is potentiated in DOCA-salt hypertensive rats [7,8], suggest that contribution of Up_4A to regulation of vasomotor tone may be altered in cardiovascular disease.

Following myocardial infarction (MI), the balance between endothelium-derived vasoactive factors is altered, even in the remote coronary vasculature; endothelial nitric oxide synthase (eNOS) uncoupling results in a decreased NO production, while the production of reactive oxygen species (ROS) is increased [9–14]. Moreover, we have previously shown that the coronary vasodilator response to ATP, which activates P1 as well as P2 receptors [5,15]

* Corresponding author at: Experimental Cardiology, Thoraxcenter, Erasmus MC, University Medical Center Rotterdam, PO Box 2040, 3000 CA Rotterdam, The Netherlands. Tel.: +31 10 7044025; fax: +31 10 7044769.
E-mail address: d.merkus@erasmusmc.nl (D. Merkus).

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and involves the activation of eNOS [16], is blunted following MI [17]. These observations suggest that purinergic receptor expression and/or signaling is altered following MI, and hence that the response of the coronary vasculature to Up_4A may be altered after MI.

Consequently, the present study aimed to determine whether the coronary vascular response to Up_4A is altered after MI, and to study possible changes in the contribution of purinergic receptor subtypes to the Up_4A responses in the coronary microcirculation following MI.

2. Methods

2.1. Animals

Studies were performed with approval of the Animal Care Committee at Erasmus Medical Center Rotterdam (NL). Fourteen Crossbred Yorkshire X Landrace swine (2–3-month-old, 25 ± 1 kg at the time of surgery) of either sex entered the study. After one week of daily adaptation to laboratory conditions, swine were sedated with an intramuscular injection of Zoletil (Tiletamine/Zolazepam; 5 mg/kg), Xylazine (2.25 mg/kg) and Atropine (1 mg), a small catheter was placed in an earvein for subsequent administration of anesthesia and fluid. Swine were intubated and ventilated with a mixture of oxygen and nitrogen (1:2, v/v). Anesthesia was induced and maintained using fentanyl (10 μg/kg/h, i.v.) [18]. Heart rate was monitored and pain reflexes were checked regularly and when necessary, additional anesthesia was administered. A thoracotomy was performed in the fourth left intercostal space. A catheter was inserted into the aorta. Subsequently, the proximal part of the LCx was exposed and occluded for 2 h followed by reperfusion to induce MI ($n = 7$), whereas no occlusion was performed in Sham ($n = 7$). Heparin (5000 IU) was administered just prior to occlusion and 1 h into occlusion to prevent clotting in the distal coronary vasculature. The aortic catheter was tunneled subcutaneously to the back and the thorax was closed in layers. Post-operative analgesia was provided by administration of Temgesic (Buprenorfine; 0.015 mg/kg i.m.) in combination with a fentanyl slow-release patch (12 μg/h) for 72 h. The catheters were flushed three times per week with 5000 IU/ml heparine.

2.2. In vivo measurements

Five weeks after induction of MI or Sham operation, all animals were sedated with Zoletil (5 mg/kg), Xylazine (2.25 mg/kg) and atropine (1 mg), anesthetized with pentobarbital (20 mg/kg/h i.v.) and artificially ventilated. A Millar high fidelity microtipped pressure catheter was inserted via the carotid artery for measurement of left ventricular pressure and a Swan Ganz catheter was inserted via the jugular vein for measurement of pulmonary artery pressure and of cardiac output via thermodilution [19]. Following thoracotomy, hearts were arrested and immediately excised and placed in cold, oxygenated Krebs bicarbonate buffer solution.

2.3. In vitro myograph studies

Coronary small arteries (diameter ≈ 150 μm) were dissected out from the apex (perfusion territory of the left anterior descending coronary artery in swine) of swine with MI and Sham-operated swine, as well as from swine hearts obtained at a local slaughterhouse and stored overnight in cold, oxygenated Krebs bicarbonate solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 8.3; pH 7.4. The next day, arteries were cut into segments of ≈2 mm length and mounted in microvascular myographs (Danish Myo Technology) with separated 6 ml organ baths containing Krebs bicarbonate solution aerated with 95% O_2/5% CO_2 and maintained at 37 °C. Changes in contractile force were recorded with a Harvard isometric transducer. Following a 30 min stabilization period, the internal diameter was set to a tension equivalent to 0.9 times the estimated diameter at 100 mmHg effective transmural pressure. The vessels were then exposed to 30 mM KCl twice. Endothelial integrity of coronary arteries was verified by observing dilation to 10 nM substance P, after preconstriction with 100 nM of the stable thromboxane A_2 analogue 9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin F2α (U46619). Then vessels were subjected to 100 mM KCl to determine the maximal vascular contraction. Thereafter, vessels were allowed to equilibrate in fresh Krebs solution for 30 min before initiating different experimental protocols [4,20]. Only one protocol was executed per vessel segment and within one protocol, all vessels were obtained from different animals.

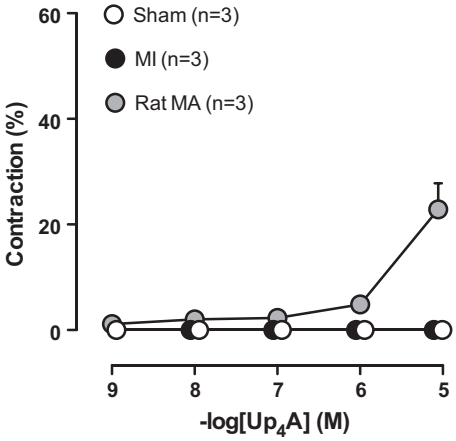

Fig. 1. Up_4A concentration-response (10^{-9}–10^{-5} M) in coronary small arteries from Sham-operated swine (Sham) and swine with a 5-week old myocardial infarction (MI) and rat mesenteric arteries (MA) under basal tone. Values are mean ± SEM.

2.3.1. Experimental protocols

Coronary small arteries from Sham-operated, slaughterhouse controls (SH-Controls) and MI swine were subjected to Up_4A (Biolog Life Science, Bremen, Germany) in incremental concentrations ranging from 10^{-9} to 10^{-5} M in the absence and presence of preconstriction with U46619. Contrary to previous observations in various vascular beds in rodents [3], but similar to our previous study in isolated porcine coronary arteries, Up_4A failed to produce vasoconstriction. To assess whether the lack of vasoconstriction was due to our experimental conditions, we subjected rat mesenteric arteries (diameter ≈ 100 μm) to the same dosages of Up_4A. Up_4A did produce vasoconstriction in these vessels (Fig. 1) that was similar in magnitude as in previous studies [8].

To assess the involvement of different purinergic receptors in the vasodilator response to Up_4A, coronary small arteries from Sham-operated and MI swine were pre-incubated with non-selective P1 receptor antagonist 8-phenyltheophylline (8PT, 10^{-5} M), non-selective P2 receptor antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS, 10^{-5} M) adenosine A_2A receptor antagonist SCH58261 (10^{-7} M), $P2X_1$ receptor antagonist MRS2159 (10^{-5} M), and $P2Y_1$ receptor antagonist MRS2179 (10^{-6} M) [4] followed by preconstriction with U46619 (100 nM). To investigate if the role of NO in the vasodilator response to Up_4A was altered after MI, vessels from both Sham-operated and MI swine were exposed to Up_4A (10^{-9}–10^{-5} M) in the absence and presence of nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-L-arginine methyl ester HCl (LNAME, 10^{-4} M) [4]. Unless otherwise mentioned, all antagonists and